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IN
ECTOCARPUS SILICULOSUS DILLW.

GEORGE FREDERIK PAPENFUSS

A DISSERTATION

SUBMITTED TO THE BOARD OF UNIVERSITY STUDIES OF THE
JOHNS HOPKINS UNIVERSITY IN CONFORMITY WITH
THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

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ALTERNATION OF GENERATIONS IN *ECTOCARPUS SILICULOSUS*¹

GEORGE F. PAPENFUSS

(WITH PLATES VI, VII AND THIRTEEN FIGURES)

Introduction

After the publication of the paper by BERTHOLD (2) in 1881, the plurilocular sporangia of *Ectocarpus siliculosus* Dillw. were regarded as gametangia and their zooids as gametes. Many investigators (21-24, 9-12, and others) found, however, that the zooids from the plurilocular sporangia of this species do not consistently function as gametes but more frequently act as zoospores. In order to explain this asexual behavior, the theory was generally advanced that the gametes had lost their sexual power and germinated parthenogenetically. The cytological work of KNIGHT (7) has proved this theory to be incorrect, for it has shown that there are two kinds of plurilocular sporangia in *E. siliculosus*: one type occurring on haploid plants and forming gametes and the other type occurring on diploid plants and giving rise to diploid zoospores which germinate into other diploid plants.

KUCKUCK (9) and REINHARDT (20) found that the zooids from the unilocular sporangium of *E. siliculosus* are zoospores while KNIGHT (7) believes that they function as gametes at Port Erin. The cytological studies of KNIGHT showed all the plants in this locality to be diploid. No reduction divisions occur in the plurilocular sporangium, and the zooids from this organ are diploid zoospores which germinate directly into other diploid plants. In the unilocular sporangium, on the contrary, the first division of the nucleus is a reduction division and the zooids formed are haploid. These zooids conjugate to form zygotes which develop into diploid plants. At Port Erin, therefore, no alternation of generations occurs; all the plants are diploid.

KNIGHT (7) studied *Ectocarpus* at Naples also, and confirmed the observations of BERTHOLD (2), OLTMANNS (18), and HARTMANN (5) that the zooids from the plurilocular sporangium are gametes in this

¹ Botanical contribution from The Johns Hopkins University, no. 127.

locality. The cytological work of KNIGHT showed, moreover, that the plants at Naples are haploid. Diploid plants are reported to be absent here. Since no reduction division occurs at the germination of the zygote in any brown alga thus far investigated, it is very probable that the zygotes at Naples develop into diploid plants.

KYLIN (13) in 1918 predicted the discovery of an alternation of generations in this species, and the cytological work of KNIGHT (7) suggests that it may occur in certain localities. It thus becomes important to know what rôle is played by the zoids from the unilocular sporangia on diploid plants, in all places where, as at Naples, the zoids from the plurilocular sporangia of haploid plants serve as gametes. Do the zoids from the unilocular sporangia in such localities act as gametes, as at Port Erin, or are they zoospores? It would be of great interest if they should function as gametes for it would show that this species has an alternation of generations and that both generations are capable of forming gametes, a unique condition among plants. Furthermore, may unilocular sporangia occur on haploid plants; and, if so, what is the function of the zoids?

It was with these problems in mind that the writer took up this study. The preliminary observations were made on plants found floating near Annapolis in the Chesapeake Bay during April, 1930. These plants bore only plurilocular sporangia and the zoids were zoospores. During the late autumn of 1930, material was collected at Cold Spring Harbor, Long Island, New York. This material also contained only plurilocular sporangia and the zoids were again asexual.

In order to obtain more abundant material than was found in the upper Chesapeake Bay, and to secure if possible plants with unilocular sporangia, this study was continued at Woods Hole, Massachusetts, over a period of three years. A brief preliminary account of the results obtained has been published (19). The present paper gives a more complete account of the functions of the different kinds of zoids, of the cytology of plants from the sea, and of the results obtained from cultural studies.²

² The following two papers on the life history of *E. siliculosus* appeared after this paper had been sent to press: FÖYN, B., Über den Lebenscyklus einiger Braunalgen. Bergens Museums Årbok. Nat. 2:1-9. 1934; SCHUSSNIG, B., and KOTHBAUER, E., Der Phasenwechsel von *Ectocarpus siliculosus*. Österr. Bot. Zeitsch. 83:81-97. 1934.

Observations on living marine plants

A. FUNCTION OF ZOIDS OF ASEXUAL PLANTS

All the observations on the function of the different kinds of zoids were made by placing pieces of *Ectocarpus* in a hanging drop and studying the behavior of the zoids liberated. A small drop of water was allowed to flow under the edge of the cover. This prevents the cover from sliding off the depression in the slide and also minimizes evaporation from the suspended drop. If it was necessary to keep a slide longer than five or six hours, the slide was placed in a damp chamber and the material in the suspended drop transferred to a fresh drop at least once a day. The water was changed usually in the morning since this is the time that liberation of zoids commonly occurs and a change of water often causes immediate liberation.

Ectocarpus siliculosus was first collected at Woods Hole during the early part of June, 1931; and was found on *Zostera* growing at Grassy Island. These plants bore only plurilocular sporangia (fig. 1) and the zoids were zoospores. Unilocular sporangia (fig. 2) were found during the third week of June on plants which were floating or which were caught on the ropes of lobster pots between Nobska Point and Nonamesset Island. The liberation of zoids from these sporangia was awaited with keen interest, for it was expected, in the light of KNIGHT'S (7) work, that the zoids would function as gametes. Contrary to such expectation, conjugation never occurred among the zoids from the unilocular sporangia, and the early germination of practically every individual zoid showed that they are zoospores.

Occasionally certain plants from the Nobska-Nonamesset region bore plurilocular as well as unilocular sporangia. The zoids from the plurilocular sporangia of such plants were also uniformly asexual. *E. siliculosus*, bearing an abundance of both unilocular and plurilocular sporangia (fig. 3) and growing as an epiphyte on *Chorda filum*, was collected later in the season at Nobska, Grassy Island, and Pine Island. During mid-summer about 50 per cent of the unilocular sporangia were parasitized by a chytrideaceous fungus; but this did not interfere with the study since many uninfected sporangia were to be had.

As plants of *E. confervoides* were found which also bore both unilocular and plurilocular sporangia simultaneously, a number of



FIGS. 1-6.—Unretouched photomicrographs showing: Fig. 1, branch of diploid plant of *E. siliculosus* bearing the plurilocular sporangia by which this species is characterized taxonomically. Material fixed in formalin acetic alcohol. Fig. 2, branch of diploid plant of same bearing unilocular sporangia only. Chromo-acetic-osmic acid material. Fig. 3, branch of diploid plant of same bearing plurilocular and unilocular sporangia on same individual. Formalin acetic alcohol material. Fig. 4, branch of *E. confervoides* with unilocular sporangia. Note that majority of unilocular sporangia are sessile in *E. confervoides* while they are usually stalked in *E. siliculosus*. Formalin material. Fig. 5, branch of *E. confervoides* bearing plurilocular sporangia. Formalin material. Fig. 6, branch of haploid plant of *E. siliculosus* bearing plurilocular organs which function as gametangia. Note that these are smaller than the plurilocular sporangia of the diploid asexual plants as shown in figs. 1, 3. Formalin material. All $\times 100$.

observations were made on the behavior of the zoids of this species. The results obtained were the same as those for *E. siliculosus*, for the zoids from both kinds of sporangia were zoospores which germinated directly. Unilocular and plurilocular sporangia occurring on separate plants of *E. confervoides* are shown in figures 4 and 5.

B. FUNCTION OF ZOIDS OF SEXUAL PLANTS

In July of 1931 *Ectocarpus siliculosus*, growing on *Chordaria flagelliformis*, was collected on the west end of Penikese Island, about 20 miles southwest of Woods Hole. These plants of *Ectocarpus* bore only plurilocular sporangia. As the material was comparatively free from diatoms, it was used for cytological work and fixed hourly during the next 24 hours. The morning of the third day after collection offered the first opportunity for studying the living plants. Although the material appeared to be in good condition, no liberation of zoids occurred and the plants finally died.

Cytological studies made the following winter showed that the plants from Penikese were haploid while the plants collected at Woods Hole proper, judging from the chromosome number in the plurilocular sporangia, were diploid. The cytology of the unilocular sporangium remained unsettled at the time.

Since no conjugation occurred between the presumably haploid zoids from the unilocular sporangia, it seemed clear that if conjugation occurred in the Woods Hole region, it must be between the zoids of the haploid plants at Penikese.

In order to determine whether the haploid plants were sexual, the writer returned to Woods Hole in August, 1932, and visited Penikese in early September. *E. siliculosus* was again found on *Chordaria* at the same locality as in the previous summer. In addition, other plants were obtained also on *Chorda* which grew mixed with the *Chordaria*. This collection presented two problems for solution: (1) Do the plants on *Chordaria* produce gametes? (2) Are the plants on *Chorda* from this locality asexual and do they bear both kinds of sporangia simultaneously, similar to those on *Chorda* at Woods Hole? If these two questions could be answered in the affirmative, it would show that an alternation of generations occurs in the life cycle of *E. siliculosus* at Penikese.

A microscopic examination showed that the plants on *Chorda* were similar to those occurring on this host at Woods Hole, in that they bore both kinds of sporangia, while the plants on *Chordaria* bore only plurilocular sporangia (fig. 6). The plants on *Chorda* liberated zoids freely the following morning and the zoids from both kinds of sporangia proved to be zoospores. It could not be determined whether the plants on *Chordaria* were sexual as no liberation of zoids occurred and the plants finally died. These plants at first appeared perfectly healthy and it was difficult to understand why no zoids were liberated, since the plants on *Chorda* which were collected at the same time and kept in similar running water aquaria liberated zoids freely.

It appeared probable that liberation of zoids might occur if the plants growing on *Chordaria* were taken directly from the ocean. On September 10, therefore, plants of *E. siliculosus* growing on *Chordaria* and *Chorda* respectively were collected at Penikese. Separate hanging-drop cultures were made of plants from each host. Pieces from several plants were placed in each culture. No liberation of zoids occurred that day but the following morning at daybreak liberation of zoids occurred in practically every drop and many conjugations were observed. The zygotes (fig. 14) were formed, however, only in the hanging drops containing plants growing on *Chordaria*. Although hundreds of zoids from both kinds of sporangia were present in the hanging drops containing plants from *Chorda*, not a single zygote was present in these drops. These observations thus showed that the haploid plants on *Chordaria* were in reality sexual while the diploid plants on *Chorda* had for two seasons proved themselves to be asexual.

An experiment performed at Cuttyhunk indicates that the previous failures to obtain liberation of zoids from the plants growing on *Chordaria* may be owing to the fact that *Ectocarpus* was left attached to the host. If *Ectocarpus* is removed from *Chordaria*, placed in dishes and the water changed twice a day, it liberates gametes and remains healthy for several days; while plants which are attached to *Chordaria* and kept under similar conditions fail to liberate gametes after the second day. *Chordaria* when kept in an aquarium exudes a gelatinous substance which may have a pathological effect on *Ectocarpus* and thus prevent the liberation of zoids.

The early part of October, 1932, was spent at Penikese in order to study the following questions with respect to the sexual plants: (1) Are the plants monoecious or dioecious? (2) Do unilocular sporangia occur on haploid plants? (3) Do the sexual plants have certain plurilocular sporangia which produce asexual spores and not gametes? (4) Are either or both female and male gametes capable of parthenogenetic development?

To determine whether the sexual plants are monoecious or dioecious, single branches were cut off under a dissecting microscope, transferred to a drop of water in a watch glass, and reexamined to make certain that only one branch was included. After a single branch had been obtained, it was washed in filtered sea water and cut into two pieces. One of the pieces was then placed in a drop of filtered sea water on a cover glass and a hanging-drop culture designated *A* was made of it. In a similar way a second hanging-drop culture *B* was made of one of the two pieces of a branch from another plant. The remaining two pieces of the respective branches were combined in a culture *AB*. In this way three hanging-drop cultures, *A*, *B*, *AB*, were obtained from two branches of separate plants of *Ectocarpus*.

In order to guard against the possible presence of stray gametes in the hanging drops, the cultures were made in the late afternoon or evening after liberation of gametes had ceased for the day. Each slide was furthermore examined under a compound microscope to insure that no gametes were present.

Cultures made in this manner gave conclusive results, for it was observed that zygotes were formed each morning only in the hanging drops which contained pieces from two separate plants, and in no instance did zygotes occur in the drops containing a piece from but one plant. It was thus decisively established that the sexual plants are dioecious. By transferring the small pieces to fresh hanging drops once or twice a day, it was possible to obtain liberation of gametes from some branches for several days.

In certain of the suspended drops containing two pieces from separate plants of *Ectocarpus*, zygotes were not formed although gametes were present. The absence of zygotes in such drops was rarely due to the fact that only one of the two pieces had liberated gametes but usually because both pieces were of the same sex. If

zygotes were formed in a drop on one day but not on another, it was proof that only one of the associated pieces had liberated gametes in the latter case. Since the female gametes can be distinguished from the male gametes by the short period of motility of the former, it is a simple matter to determine whether the absence of zygotes in a hanging-drop culture containing two pieces from separate plants is due to the pieces being of the same sex. Furthermore, a microscopic examination of the gametes in the two drops which contain the other half of the respective branches will show that the gametes in both drops are of the same sex. The sex of the gametes in a drop containing pieces of two branches from separate plants can be determined also by experiment as follows: Gametes were present in the cultures *G*, *GH*, and *H*, but none of these contained zygotes. The gametes were all female, judging from the short period of motility. The following morning gametes were again liberated in the three respective cultures and the gametes from a branch *E*, which had previously shown itself to be male, were added by means of a small pipette to the cultures *G* and *H*. After a few minutes, conjugation commenced in these two drops while no fusions occurred in the drop *GH*. Thus it was conclusively proved that the gametes from the branches *G* and *H* were female.

From the writer's observations there is no indication of a "relative sexuality" such as HARTMANN (5) found at Naples, but the evidence at hand is not sufficient to preclude the possibility of its occurrence.

The assembling of large numbers of male gametes around a female as figured by BERTHOLD (2) was not observed. In no instance did more than about six male gametes gather around a female simultaneously, and in many cases the female seemed to attract only one male. Conjugation was not observed to occur while a female gamete was still swarming but always took place after the female had become attached at the edge of the drop toward the source of light.

The actual fusion of gametes is a rapid process which at most does not last longer than 20 seconds; and the successive stages of conjugation of any two gametes, as are figured by BERTHOLD (2), follow each other so rapidly that they cannot be observed in the living state. But it was found that the process of fusion is greatly retarded if male gametes are added to a culture of female gametes which has been

liberated for about one hour. After this lapse of time, a firmer membrane has been formed around the female gamete, and as a result the coalescence of conjugants is accomplished more slowly. Under these conditions a male gamete may attach itself to a female gamete, perform the usual vibratory movements, and then suddenly move toward the latter as in normal conjugation; but owing to the thickened membrane of the female a coalescence does not occur and the male gamete immediately settles down motionless beside the female. It is evident that the chances of fertilization are greatly reduced by the thickening of the membrane which renders the female gamete incapable of fertilization not long after liberation. It is not known how long after liberation a male gamete remains functional.

The male gamete usually unites with the broad posterior end of the female, as was observed by BERTHOLD (2) and KUCKUCK (12). This is undoubtedly due to the fact, as BERTHOLD and KUCKUCK state, that the attached anterior end of the female is not accessible to the male.

There is considerable variation in size among the gametes of each sex, some being nearly twice as large as others. Large and small gametes arise from different gametangia respectively. At first it was thought that these large zoids were zoospores which serve to propagate the haploid generation; but it was later observed that the larger as well as the smaller zoids from the sexual plants function as gametes. In consequence zygotes of various sizes are formed: large, small, and intermediate ones, depending upon the conjugation of two large gametes, of two small gametes, or of a large with a small gamete.

Unilocular sporangia were not found on the sexual plants, and the only means by which these plants can propagate themselves is by the parthenogenetic development of the gametes of either sex, as will be discussed later.

C. MISCELLANEOUS OBSERVATIONS ON ASEQUAL AND SEXUAL PLANTS

MORPHOLOGY.—Asexual plants are more robust than sexual plants (fig. 15) and frequently attain a length of more than 7 cm., while the largest sexual plants collected were but 4 cm. long. The

cells of asexual plants are considerably larger than those of sexual plants and measure on an average $50 \times 70 \mu$ while the cells of sexual plants average only $44.5 \times 45.5 \mu$. The primary difference between the cells of asexual and sexual plants is thus in the length of the cells. The breadth of many cells of the sexual plants exceeds the length, a condition less common in the asexual plants. The plurilocular sporangia of asexual plants (fig. 3) are considerably larger and vary more in size and form than those of sexual plants (fig. 6). The familiar ropelike intertwining of the main axes is also more characteristic of the robust asexual plants.

DISTRIBUTION.—Sexual plants are of limited distribution and were found only at Penikese Island where they occur in large quantities and exclusively on *Chordaria*. Asexual plants are more widespread and were collected on *Spartina* and *Chorda* at Woods Hole, on *Chorda* and *Chordaria* at Cuttyhunk, and on *Chorda* at Penikese. It is a striking fact that the asexual and the sexual plants grow side by side at the latter locality but are each confined to its own host.

DISCHARGE OF ZOIDS.—The liberation of zoids, especially of the sexual plants, occurs primarily during the early morning and forenoon; but if the plants are kept in darkness overnight and placed in fresh sea water when exposed to daylight, they can often be induced to liberate zoids at other times of the day. As a rule, the opening of the plurilocular sporangium occurs at the apex. The opening of the unilocular sporangium is always at the apex.

It was not observed that the content of the unilocular sporangium is expelled in bulk as was found by KNIGHT (7). The zoids are contained in a gelatinous mass which oozes out of the sporangium, carrying the zoids with it. As the mass increases in size on the exterior of the sporangium, stages such as are shown in KNIGHT'S figures 33–35 may be observed. In many instances the perforation is very small and each zoid becomes constricted to a dumb-bell shape as it passes through the pore. After liberation, the zoids remain in a spherical mass at the apex of the sporangium for a short time, then become free and swim off individually. The process of liberation frequently consumes more than two minutes and at times some of the escaped zoids have become motile while others are still within the sporangium. These observations on the liberation of zoids from

the unilocular sporangium were made on plants cultured in aquaria, and it is possible that this environment altered the normal mechanics of discharge.

As many as thirty-two zoids were counted from a unilocular sporangium of a plant from the sea while counts of four, eight, and sixteen zoids were made from the unilocular sporangia of plants grown in aquaria. The unfavorable environmental conditions of an aquarium have a dwarfing effect on the plants and their reproductive organs. It is doubtful whether unilocular sporangia from the sea contain fewer than thirty-two zoids and it is possible that as many as sixty-four may be formed in certain very large unilocular sporangia.

PERIOD OF MOTILITY OF ZOIDS.—Female gametes have the shortest swarming period of all the zoids, and frequently become stationary at the edge of a hanging drop five minutes after liberation; about 15 minutes is their maximum period of motility. Male gametes, on the contrary, have the longest swarming period of all the zoids and at times are still motile eight hours after liberation. Zoids from the plurilocular sporangia of asexual plants often remain motile for 3–5 hours while the zoids from the unilocular sporangia seldom remain motile for more than 30 minutes.

PHOTOTACTIC RESPONSE OF ZOIDS.—Although the great majority of all zoids are positively phototactic, a certain proportion react either negatively or as “neutrals.” No adequate explanation can be given for this difference of response, but certain observations suggest that a disturbance of the zoids during their swarming period may stimulate them to become negatively phototactic. If positively phototactic zoids are taken from a vessel and placed in a hanging drop, a large proportion of them become negatively phototactic. (KYLIN (14) has also found this to be true.) If, however, hanging-drop cultures are made in the evening from pieces of *Ectocarpus* and these cultures placed in a dark room with light entering only through a small window, practically 100 per cent of the zoids liberated the following morning are positively phototactic.

SIZE OF ZOIDS.—A considerable difference in size exists among the three types of zoids. The zoospores from the unilocular sporangium are large and measure on an average $9.1 \times 18 \mu$; the gametes are small

and measure on an average only $4 \times 7.4 \mu$; and the zoospores from plurilocular sporangia of asexual plants are intermediate in size and measure on an average $4.8 \times 9.5 \mu$. Large zoids from unilocular sporangia and smaller zoids from diploid plurilocular sporangia are shown in figures 16–18. The difference in size between the gametes and the asexual zoids may be seen in figures 14, 16, 17, and 18.

SEASONAL CYCLE.—Although the writer's data regarding the seasonal cycle of *E. siliculosus* are not entirely complete, certain facts are worth recording. At Woods Hole, in contrast to the several localities where previous investigators have worked, unilocular sporangia are common and can be found throughout the summer months. Plants bearing unilocular sporangia were collected for the first time during the third week of June, 1931; but since these plants contained many mature and empty unilocular sporangia, there is reason to believe that these organs had begun to appear earlier in the year. Unilocular sporangia evidently disappear in autumn at Woods Hole and remain absent during the winter. Plants collected at Woods Hole on October 30, 1931, bore very few of these organs but many plurilocular sporangia. (*E. siliculosus* was rare at this time of the year.) The latest collection of the year was obtained from a small amount of material received from Woods Hole on December 21, 1931. These plants bore many plurilocular sporangia similar to those shown in figure 1 but had no unilocular sporangia. Although unilocular sporangia were abundant on *Ectocarpus* at Penikese during the early part of September, 1932, they had disappeared completely by the first week of October of that year. During the latter half of September, 1933, unilocular sporangia were found at Woods Hole on two occasions only; and they were entirely absent on plants received from Woods Hole on November 27, 1933.

The evidence from three seasons' collecting thus indicates that unilocular sporangia occur in the Woods Hole region primarily during the summer months while diploid plurilocular sporangia occurred at all seasons at which collections were made (June 5 to December 21). It is possible that the asexual plants occur throughout the year at Woods Hole and that their rareness during winter is owing to the transitory habit of the hosts.

The available data on the seasonal occurrence of sexual plants

are too fragmentary to justify any conclusions. Plants with gametangia were first collected in July, 1931, again in September of 1931 and of 1933; and they were still abundant during the first two weeks of October, 1932.

Cytology

FIXING.—The following fixing agents gave good results: (1) CHAMBERLAIN'S (3) chromo-acetic-osmic acid solution which consists of 97 parts sea water, 1 part chromic acid, 1 part glacial acetic acid, and 1 part of 1 per cent osmic acid. The time of fixation required is four to five minutes. (2) Allen's modifications of Bouin's fluid (17): (a) B-15, which is the original Bouin's fluid made up with sea water to which is added 2 parts of urea and 1.5 parts of chromic acid; (b) B-3, which consists of picric acid, saturated solution in sea water, 75 parts; formalin 15 parts; glacial acetic acid 10 parts; and chromic acid 1 part. The time of fixation required is one to two hours. Mitoses are more abundant if the material to be fixed is first kept in darkness for two or three hours, as is suggested by DAMMANN (4).

DEHYDRATING.—Taylor's method (17) for dehydration was followed with minor modifications. The material was left one-half to one hour in each grade of the alcohol, salt water, and fresh water, indicated in table I, and was allowed to remain in 70 per cent alcohol until dehydrated for imbedding.

In making paraffin sections, the trouble caused by epiphytic diatoms may be partly overcome if the material to be sectioned is treated for 24 hours with a 5 per cent solution of hydrofluoric acid in 70 per cent alcohol, and subsequently washed in 70 per cent alcohol.

SECTIONING AND STAINING.—The usual methods of clearing, infiltration, and imbedding were followed. By placing the material selected for sectioning in a small cloth bag and transferring the bag from grade to grade of the alcohols and xylols, much time is saved. The material is taken out of the bag when it is in the final xylol. Rubber tubing rings placed on glass slides coated with glycerine, as described by BAUMGARTNER and WELCH (1), were used as imbedding troughs.

The sections were cut 3-4 μ thick. Since a few diatoms always remain attached to the material, a great deal of time lost in sharpening the knife can be avoided by using Wade and Butcher safety blades and the microtome blade holder of the same make.

The material fixed in chromo-acetic-osmic acid was bleached in a 5 per cent aqueous solution of hydrogen peroxide for one hour.

Feulgen's reaction (LEE 15, pp. 306-307, 437-438; MARGOLENA 16; KRAUSE 8, p. 1792; WESTBROOK 28) and Haidenhain's iron-alum

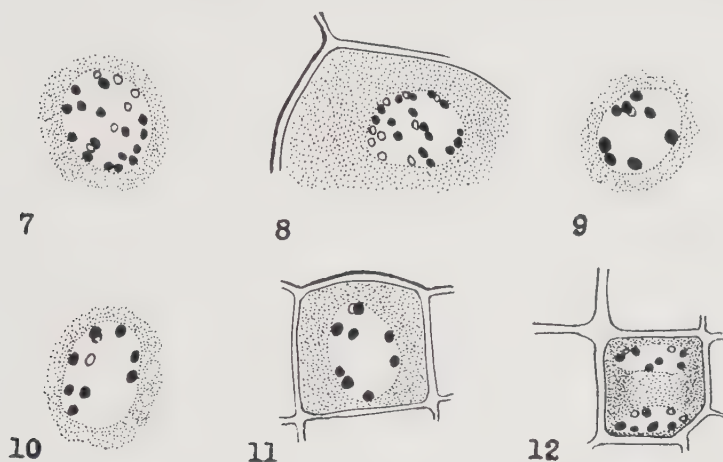
TABLE I

WATER, FRESH (PARTS)	WATER, SALT (PARTS)	ALCOHOL, 95 PER CENT (PARTS)
1	93	1
2	91	2
3	89	3
4	87	4
5	85	5
6	83	6
7	81	7
8	79	8
9	77	9
10	75	10
12 $\frac{1}{2}$	70	12 $\frac{1}{2}$
15	65	15
20	57 $\frac{1}{2}$	17 $\frac{1}{2}$
25	50	20
30	40	25
35	30	30
40	15	40
45		50
35		60
25		70

haematoxylin were used as stains, but Feulgen's reaction gave the more satisfactory results. All the mitotic figures are drawn from preparations stained by this method. The chief advantage of Feulgen's reaction lies in the fact that, with correct treatment, it is practically specific for chromatin; and it leaves nucleoli and cytoplasmic inclusions unstained. The cytoplasm appears perfectly homogeneous and clear, especially if the material is fixed with B-15 or B-3. In this study the sections were treated with the 0.5 normal HCl for ten minutes and stained in the fuchsin sulphurous acid for four hours.

CHROMOSOMES. -The primary objective of this phase of the work was to determine whether the chromosomal cycle coincides with the

morphological phases of the life cycle. Although the results are incomplete in certain details, it has nevertheless been possible to establish that: (1) The asexual plants, with both plurilocular and unilocular sporangia, are diploid. No chromosome reduction occurs in the plurilocular sporangium and the nuclei in this organ contain about sixteen chromosomes (figs. 7, 8). In the unilocular sporangium on the contrary, one of the early nuclear divisions is undoubtedly a reduction division, for the nuclei which are formed later contain but



FIGS. 7-12.—Allen's fixative; Feulgen's reaction. Camera lucida drawings from paraffin sections: Fig. 7, polar view of early anaphase in plurilocular sporangium of asexual plant showing at upper level diploid number of chromosomes (16) and at a lower level 6 (unshaded) chromosomes of set for opposite pole. Fig. 8, anaphase from another plurilocular sporangium showing 16 chromosomes at upper level and 9 (unshaded) chromosomes of set for opposite pole. Fig. 9, polar view of metaphase in a unilocular sporangium showing haploid number of chromosomes (8 or 9), eight at upper level and one at lower level. Fig. 10, similar view, but from a different unilocular sporangium, showing haploid number of chromosomes (8) at upper level and two additional ones, probably of set for opposite pole, at lower level. Fig. 11, polar view of metaphase in a plurilocular sporangium of sexual plant showing haploid number of chromosomes (8 or 9); one is at a lower level than the other eight. Fig. 12, profile view of late anaphase or early telophase in a plurilocular sporangium of another sexual plant, showing haploid number of chromosomes (8) at each pole. All $\times 4320$.

eight or nine chromosomes (figs. 9, 10). (2) The sexual plants are haploid, the nuclei in the plurilocular sporangia of these plants containing eight or nine chromosomes (figs. 11, 12). Cytological obser-

vations thus show that there is an alternation of generations in the life cycle of *E. siliculosus* growing in the region of Woods Hole.

Cultures

A. AQUARIUM CULTURES.—Two-liter battery jars were employed as aquaria, and these aerated by the aid of aspirators. The aqua-

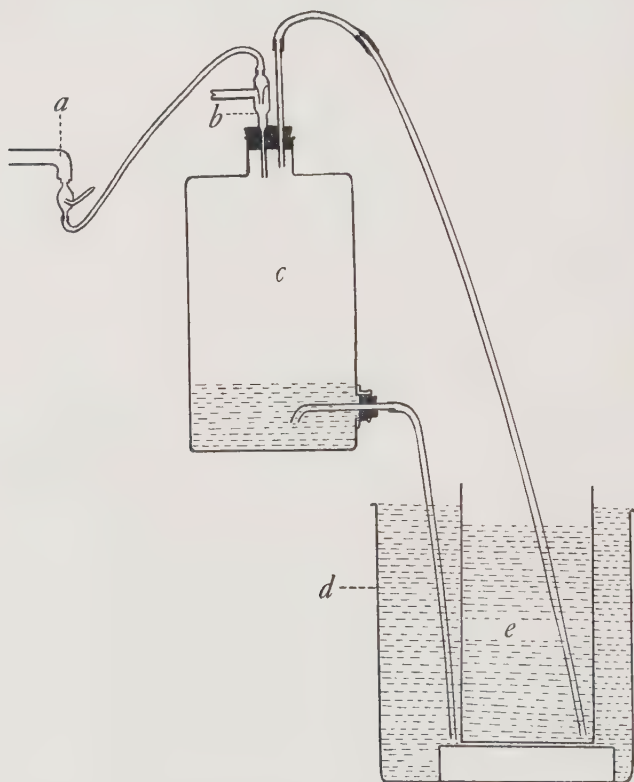


FIG. 13.—Diagram of apparatus for aerating an aquarium: *a*, faucet; *b*, filter pump; *c*, aspirator bottle; *d*, fresh water jar; *e*, salt water aquarium.

rium was kept cool by placing it within a larger jar and allowing the outflow of water from the aspirator bottle to run into the latter. Figure 13 illustrates the aerating system. The filter pump may be attached either to the faucet or, as in this case, to the aspirator bottle. Care has to be exercised not to place too much material in an

aquarium of this size. The best results are obtained by employing several aerating systems. As the sea-water evaporates from the aquarium, distilled water should be added to prevent the sea water from becoming too saline.

By thus aerating and cooling the aquarium, it was possible to grow *Ectocarpus* for two months in the laboratory at The Johns Hopkins University; and the plants undoubtedly would have survived for a longer time if small crustaceans had not devoured them. Reproductive organs continue to be formed on plants growing in aquaria but they as well as the newly formed branches become dwarfed after a short time.

It was found that many unilocular sporangia are formed on asexual plants kept in aquaria. Plants collected at Woods Hole on October 30, 1931, bore but few unilocular sporangia and many plurilocular sporangia. After a week in the aquaria, these plants started to produce many unilocular sporangia while the plurilocular sporangia decreased in number, and after 14 days these plants bore decidedly more unilocular than plurilocular sporangia. Likewise, asexual plants which were collected at Penikese in October, 1932, and which contained no unilocular sporangia, started to produce these organs after they had been in an aquarium for two weeks. Sexual plants collected at the same time and kept in a similar aquarium failed to produce unilocular sporangia, a fact which gives additional evidence that unilocular sporangia are organs confined to the diploid asexual generation, as already discussed.

B. SILICA-GEL CULTURES.—Silica gel (26, 27) is a medium which deserves to be used more generally in the culturing of algae. The inorganic composition of this gel renders it superior to all other solid media in that it supplies no nourishment to the bacteria which are inevitably introduced into a medium with the spores of the alga to be cultured.

The following simple method for preparing the gel was shown to the writer by the Reverend A. KEEFE: Equal parts of sodium silicate, sp. gr. 1.1, and hydrochloric acid, sp. gr. 1.09, are mixed by stirring and the solution poured into petri dishes. The dishes are allowed to remain undisturbed for several hours, or preferably overnight, for the gel to set, then placed in a vessel and washed in running

fresh water for at least 24 hours to remove the chlorides. In order to have the gel saturated with sea water the dishes are left in a vessel containing filtered sea water for about 36 hours. The plates are then ready for inoculation or may be autoclaved before they are used.

It is necessary to add a liquid medium to silica gel plates since the gel dries within a comparatively short time. The liquid culture medium employed so successfully by SCHREIBER (25) for the culturing of *Laminaria* gametophytes was used. This liquid medium consists of:

NaNO ₃	0.1 gm.
Na ₂ HPO ₄	0.02 gm.
Distilled H ₂ O.....	50.0 cc.
Sea H ₂ O.....	1000.0 cc.

Although plants have been raised from the spore to the formation of reproductive organs by this method, the writer has not yet obtained the entirely satisfactory results which may be expected from further experiment with this culture medium. A major source of trouble has been the rapid multiplication of diatoms which are introduced into the plates with the zoids.

C. HANGING-DROP CULTURES.³—The best cultural results were obtained from hanging-drop cultures. This method of culturing zoids is similar to the one already described for the observations on the behavior of zoids, with the exception that the parent plants are removed from the drops after liberation of the zoids has occurred and the slides are then placed in a damp chamber. The attached zoids adhere firmly to the glass, and it is possible to wash the covers by squirting sea water on them from a pipette, and thus rid them of most of the diatoms which are introduced with the parent plants.

It is necessary to dry the slides and covers frequently with filter paper, since the condensed vapor which accumulates on them tends to float up the cover slips and to flow into the hanging drops. Since the young plants adhere to the cover glass, it is possible to withdraw the drop of water with filter paper and replace it with fresh sea water from time to time without danger of losing the plantlets. In many cases the drop of sea water was replaced with SCHREIBER'S (25) culture fluid. Although diatoms multiplied rapidly in the silica gel

³ A more satisfactory method for culturing algae is described by KYLIN (14) in a paper which appeared after this study had been completed.

cultures they never constituted a serious problem in the hanging-drop cultures. By the hanging-drop method plants were grown successfully from diploid zoospores, zygotes, parthenogenetic gametes, and haploid zoospores.

Diploid zoospores usually germinate within two to five hours after they have become attached. A series of early developmental stages of plants from these zoids is shown in figures 26-32. The plants shown in figure 19 have formed plurilocular sporangia. These plants previously also bore unilocular sporangia but unfortunately no photograph was taken of these earlier sporangia. In one case mature plurilocular sporangia were present on a 45-day old plant. In the majority of cultures, however, the young plants did not produce reproductive organs within the first two months. The F_1 generation of plants from diploid zoospores is shown in figure 20. Plants grown in hanging-drop cultures are small but otherwise appear perfectly normal. Some of the plants were still in perfect condition when six months old.

The zygotes occasionally begin to germinate after nine hours but usually not before two or three days. The two eye-spots are often still visible after 48 hours. The sporelings from these structures develop very slowly as contrasted with those from diploid zoospores.

Only about 5 per cent of the unfused male and female gametes develop parthenogenetically. In a few cultures about 50 per cent of the unfertilized gametes germinated and it is possible that in the ocean considerably more than 5 per cent of the unfused gametes develop parthenogenetically. The parthenogenetic gametes are very slow in germinating. Occasionally the germ tube is discernible after 24 hours but more commonly it is 36 or 48 hours, or even more, before germination begins. The unfused gametes frequently enlarge to about twice their original size before they germinate.

The haploid zoospores from the unilocular sporangium usually begin to germinate within two to three hours after liberation. After eight hours the germ tube may be one-half the length of the zoid and after 24 hours the sporeling may have become a two-celled filament. Considerable variation in the rate of development exists, however, between individual sporelings. A series of developmental stages of plantlets from haploid zoospores is shown in figures 21-25.

After the cultures of plants from zygotes, parthenogenetic male

and female gametes, and haploid zoospores had been grown for two months without fruiting, they were accidentally allowed to dry up. Well developed vegetative plantlets were obtained from these cultures, however, and it can be concluded that zygotes, parthenogenetic gametes, and haploid zoospores, as well as the diploid zoospores, all play an active rôle in the life cycle of *E. siliculosus*.

From the observations on germination, it is evident that the initial growth of a sporeling always occurs from a germ tube arising at the narrow anterior end of a zoid, and after this tube has become several-celled, a second filament arises at the posterior end of the zoid (figs. 24, 25). A zoid always becomes attached in such a way that the narrow end is directed toward the incident light; and it seemed possible that the origin of the germ tube at this end of the zoid may be due to a photic stimulus. In order to test this, hanging-drop preparations were allowed to liberate zoids in a dark room lighted only from a very small window. After the zoids had settled at the edge of the drop toward the light, each with its narrow anterior end directed toward the window, the slides were turned through 180° and placed in a damp chamber in front of the window. Under these conditions the anterior end of the zoids, now directed away from the light, still continued to give rise to the initial germ tube. The position of the germ tube is thus independent of the direction of the incident light. Whether the place of origin of the germ tube is determined by a contact stimulus or by the polarity of the zoid is not known. In zygotes the germ tube arises from the anterior end of the female gamete. The further development of the small plants shows that they are positively phototropic, however, for the majority of filaments bend and grow toward the light. Figure 33 shows part of a hanging-drop culture treated in this way. The majority of filaments are growing toward the light and away from the edge of the drop. Figure 34, from the same culture but taken at the edge of the drop which is toward the light, shows that practically every filament is growing toward the light.

Discussion

The results of this investigation show that there is an alternation of generations in the life cycle of *Ectocarpus siliculosus* in the region

of Woods Hole. The observations of three summers have shown, however, that there is a marked difference in the distribution of the two generations. Both generations grow at Penikese Island, 20 miles from Woods Hole, while only asexual plants occur at Woods Hole itself. Since large numbers of haploid zooids from unilocular sporangia are present at Woods Hole, the absence of sexual plants in this locality becomes a matter of considerable interest. The explanation of this condition was not forthcoming until the sexual plants were found at Penikese and it was observed that they are obligate epiphytes on *Chordaria flagelliformis*, an alga which does not occur at Woods Hole. The environment at Woods Hole is unfavorable for *Chordaria*, which usually grows in exposed localities like the one at Penikese.

The asexual plants of *Ectocarpus* occur at Penikese only on *Chorda filum* which grows mixed with *Chordaria*. This would seemingly indicate that the asexual plants are obligate epiphytes on *Chorda*. This is not the case, however, for these plants occur also on *Spartina* at Woods Hole and were found on one occasion on a small amount of *Chordaria* collected at Cuttyhunk Island.

It is not known whether the condition in the region of Woods Hole also applies to other localities, since the previous investigators, who found the sexual plants of *E. siliculosus*, do not specify the host. The dependence of the sexual generation on a specific host is not unique for *Ectocarpus*, however, but is also true for *Pylaiella littoralis*. KNIGHT (6) found that the diploid plants of *Pylaiella* occur on both *Fucus* and *Ascophyllum* while the haploid plants are confined to *Ascophyllum*, even when it grows mixed with *Fucus*. (KYLIN (14) found that the haploid plants of *Pylaiella* do not grow directly on *Ascophyllum* but on *Sertularia pumila* which is attached to the latter.) It is possible that the dependence of the sexual generation on a certain host is a phenomenon of more common occurrence in algae than has been recognized.

The absence of the sexual generation of *Ectocarpus* in certain localities is due, undoubtedly, to an effect of the environment which inhibits the formation of unilocular sporangia. This is the case, apparently, at Kristineberg, Sweden, where there are only asexual plants which propagate themselves by means of zoospores from the

plurilocular sporangia (14). It is probable that a very slight difference in the environment may determine the absence of unilocular sporangia, for the writer found that asexual plants growing on *Spartina* at Grassy Island bore plurilocular sporangia only, while the plants which grew on *Chorda* in the same locality but in slightly deeper water bore both plurilocular and unilocular sporangia.

Since *Ectocarpus* has an alternation of generations in the region of Woods Hole, we would expect this to be true in other localities also, especially at Naples where sexual plants are abundant. Although there is as yet no record of an occurrence of asexual plants at Naples (except the plantlet shown in BERTHOLD'S (2) figure 8), it is evident that such plants exist there. Both BERTHOLD (2) and OLTMANNS (18) observed "neutral" spores from plurilocular sporangia, and OLTMANNS states that these are generally larger than the gametes, facts which indicate that these zoids were diploid zoospores. It is obvious that REINHARDT (20), SAUVAGEAU (22), and KUCKUCK (12) also had both generations of *E. siliculosus* at Sevastopol, Guéthary, and Helgoland respectively.

The plants investigated by KNIGHT (7) at Port Erin are similar to those occurring at Woods Hole proper, but the zoids from the unilocular sporangium behave entirely differently in the two localities, in that they function as zoospores at Woods Hole while they act as gametes at Port Erin. It is possible that the cases which KNIGHT designated as conjugations were abnormal fusions or sister zoids that had not become separated from each other in the sporangium. The following facts suggest that KNIGHT misinterpreted abnormal structures for conjugations: (1) fusions were not abundant; (2) conjugation occurred while the zoids were motile; (3) the process of fusion required about 20 minutes; (4) at times the zoids fused in groups. In contrast to this the writer found that many conjugations occur between the gametes from the plurilocular sporangia of haploid plants; conjugation always occurs between a motile male gamete and an attached female gamete; the process of fusion requires less than one-half minute; and the gametes never fuse in clumps.

KNIGHT believes that the unfused zoids from the unilocular sporangium die at Port Erin, but judging from the results obtained

at Woods Hole it may be concluded that the great majority of these zoids are capable of development into haploid plants. The absence of haploid plants at Port Erin may be due to the absence of the essential host as is the case at Woods Hole proper.

Summary

1. An alternation of generations occurs in the life cycle of *Ectocarpus siliculosus* growing in the region of Woods Hole.

2. The diploid asexual plants bear unilocular and plurilocular sporangia on separate individuals or simultaneously on the same individual. In the plurilocular sporangium are formed diploid zoospores which germinate directly into other asexual plants.

3. A reduction division occurs in the unilocular sporangium and the haploid zoids formed in this organ germinate into haploid sexual plants.

4. The sexual plants are dioecious and produce physiologically anisogamous gametes in plurilocular sporangia. The zygotes develop into diploid asexual plants.

5. The sexual generation propagates itself by the parthenogenetic development of about 5 per cent of the unfused gametes of either sex.

6. Haploid plants are inferior to diploid plants in stature, size of cells, and size of plurilocular sporangia.

7. Plants were grown in suspended drops from spore to spore. Some cultures lived for more than six months.

8. Sexual plants were found only at Penikese Island where they occur on *Chordaria*. Asexual plants are widely distributed in the region of Woods Hole and occur on *Chorda*, *Chordaria*, and *Spartina*.

9. It is suggested that the sexual plants are obligate epiphytes on *Chordaria* and that their absence at Woods Hole proper is owing to the absence of this host in this locality.

This study was undertaken at the suggestion of Professor DUNCAN S. JOHNSON, to whom the writer is greatly indebted for invaluable direction. The writer further wishes to thank Professors I. F. LEWIS and W. R. TAYLOR for helpful suggestions while the experimental work was in progress and to express his appreciation to Professor

M. H. JACOBS, Director of the Marine Biological Laboratory, Woods Hole, for granting the facilities of the laboratory for part of the summers of 1932 and 1933. The Supply Department of the Marine Biological Laboratory freely assisted in the collecting of material; and Dr. W. G. LYNN, MESSRS. G. C. KLINGEL, W. L. DOYLE, and D. B. LAWRENCE gave valuable aid in the preparation of the photographs.

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EXPLANATION OF PLATES VI, VII

All figures are unretouched photomicrographs of *E. siliculosus*.

PLATE VI

FIG. 14.—Zygotes (z) and unconjugated gametes (g). Note larger size and two eyespots of zygotes. Living hanging-drop culture. $\times 504$.

FIG. 15.—Asexual (2x) and sexual plants (x). Note difference in size. Formalin material. $\times 7/10$ natural size.

FIG. 16.—Haploid and diploid zoospores, formed in unilocular and plurilocular sporangia respectively, of diploid asexual plants. Three large zooids are from unilocular sporangium. Living hanging-drop culture. $\times 352$.

FIG. 17.—Same but from a different culture. Ten large zoids from unilocular sporangium and two small zoids on left from plurilocular sporangium. $\times 352$.

FIG. 18.—Same but from a different culture, 5 hours after liberation of zoids. Two of the seven large haploid zoids from unilocular sporangium have already germinated. $\times 332$.

FIG. 19.—Plants from diploid zoospores bearing plurilocular sporangia. Living hanging-drop culture, 3 $1/2$ months old. $\times 70$.

FIG. 20.—Living F_1 plantlets from zoospores of plurilocular sporangia formed on plants raised in hanging-drop culture from diploid zoospores. $\times 184$.

FIG. 21.—Living young plants from haploid zoospores. Hanging-drop culture, 3 days old. $\times 344$.

FIG. 22.—Living plantlets from haploid zoospores. Hanging-drop culture, 16 days old. $\times 344$.

FIG. 23.—Same, 21 days old. $\times 352$.

FIG. 24.—A 32-day old plantlet from a haploid zoospore. Living hanging-drop culture. $\times 344$.

FIG. 25.—Another plant from same culture and of same age. $\times 344$.

PLATE VII

FIG. 26.—Diploid zoospores, 10 hours after liberation: *c*, early stage in germination. Living hanging-drop culture. $\times 384$.

FIG. 27.—Same culture, 57 hours old. $\times 384$.

FIG. 28.—Same culture, 5 days old. $\times 384$.

FIG. 29.—Same culture, 13 days old. $\times 374$.

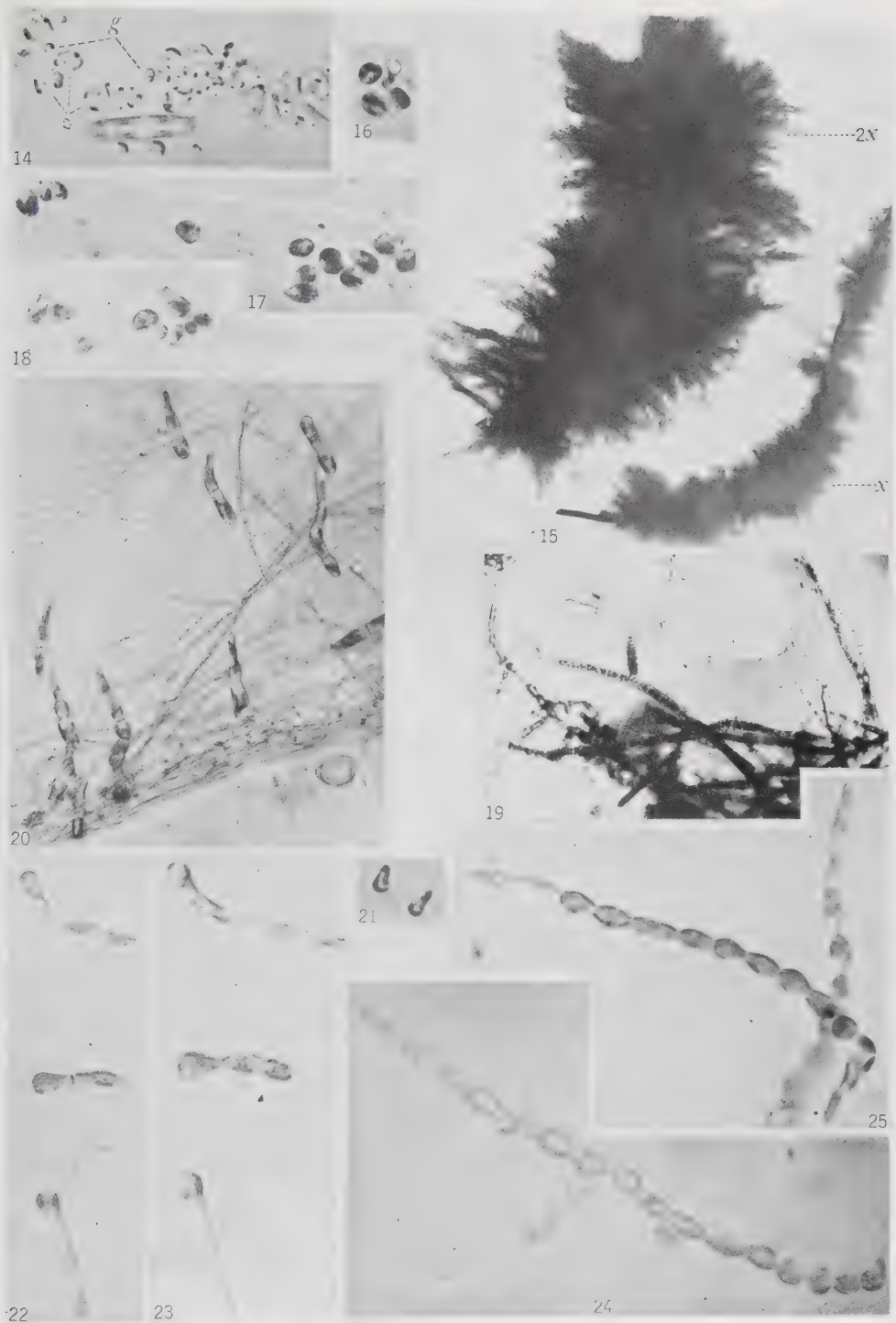
FIG. 30.—Living 14-day old plantlets from diploid zoospores. Hanging-drop culture. $\times 80$.

FIG. 31.—Living 22-day old plantlets from diploid zoospores. Hanging-drop culture. $\times 378$.

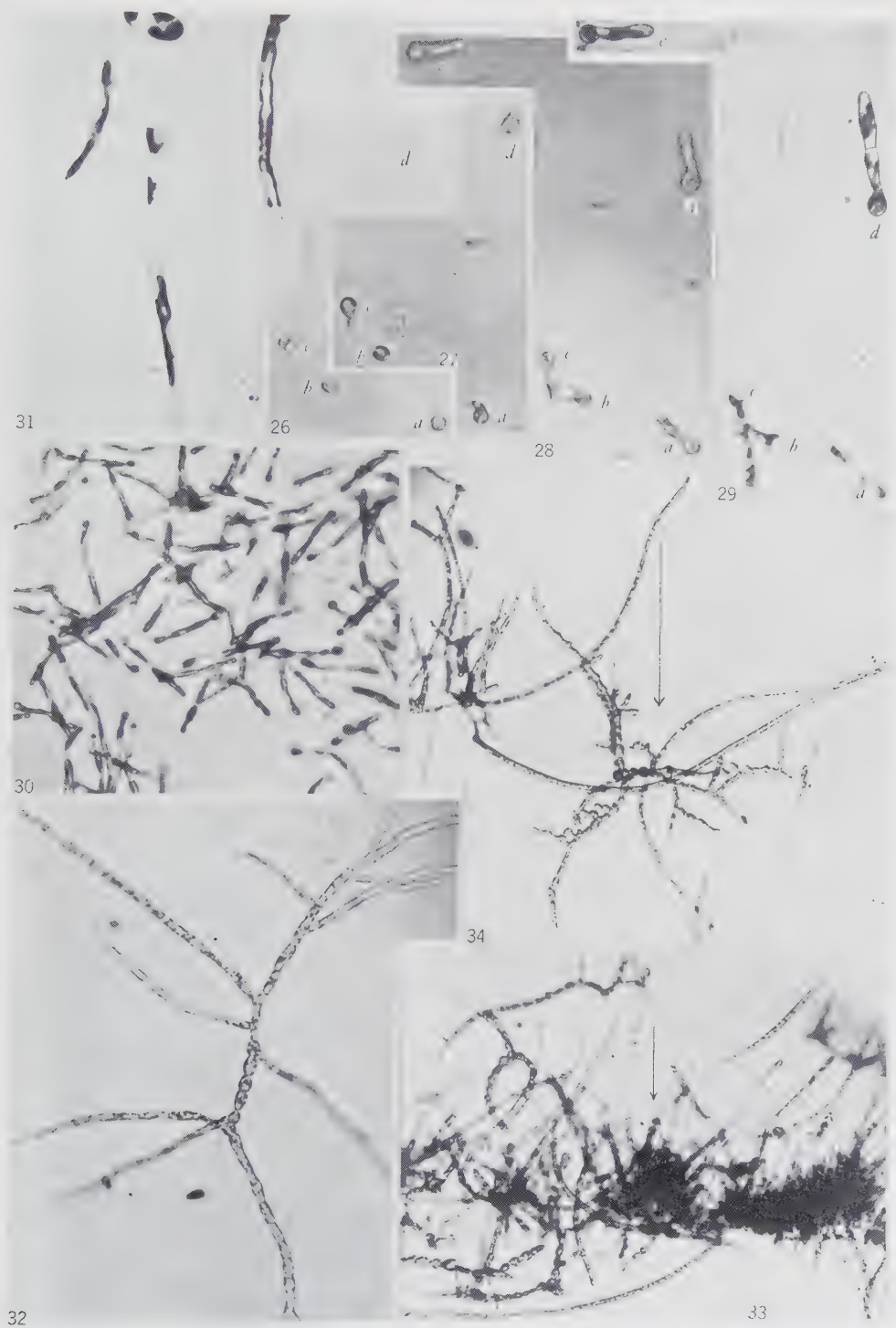
FIG. 32.—Living 30-day old plantlet from diploid zoospore. This sporeling developed unusually rapidly. Hanging-drop culture. $\times 152$.

FIG. 33.—Darker side of a 44-day old hanging-drop culture from diploid zoospores. This culture was kept in a dark room with light entering through a small window. After the zoids had become attached at edge of drop facing window, the slide was turned through 180° . The filaments are growing away from the edge of the drop and toward the source of light (indicated by arrow). $\times 68$.

FIG. 34.—Illuminated side of same culture. The filaments are growing toward the edge of the drop (toward source of light). $\times 68$.



PAPENFUSS on ECTOCARPUS



PAPENFUSS on ECTOCARPUS

VITA

GEORGE FREDERIK PAPENFUSS was born at Harrismith, Orange Free State, Union of South Africa, on November 4, 1903. He received his elementary education at the boarding school Arbeid Adelt, his secondary education at Kestell, and his high school education at Harrismith and Stellenbosch. He passed his Matriculation examination in December, 1924, and attended the University of Cape Town for the first half of the year 1925. In 1926 he came to the United States to study agriculture and entered North Carolina State College of Agriculture and Engineering in August of that year. He completed the requirements for the B.S. degree in Botany at that college in December, 1928. In January, 1929, he entered The Johns Hopkins University as a graduate student assistant in the Department of Botany, a position which he occupied until June, 1931. He was a University Scholar for the year 1931-1932; and the Ella E. Slack Scholar for the year 1932-1933. The summer of 1929 was spent at the Mount Desert Island Biological Laboratory, Maine, in a study of the marine and fresh water algae. The summer of 1931 was spent in investigation at the Marine Biological Laboratory, Woods Hole, Massachusetts. The first half of the summer of 1932 was spent in Jamaica, B. W. I., as a member of The Johns Hopkins Botanical Expedition. The remainder of that summer was spent in investigation at the Marine Biological Laboratory, Woods Hole, Massachusetts.

